

## RESEARCH COMMUNICATION

**Glutathione transferases catalyse the detoxication of oxidized metabolites (*o*-quinones) of catecholamines and may serve as an antioxidant system preventing degenerative cellular processes**

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*o*-Quinones are physiological oxidation products of catecholamines that contribute to redox cycling, toxicity and apoptosis, i.e. the neurodegenerative processes underlying Parkinson's disease and schizophrenia. The present study shows that the cyclized *o*-quinones aminochrome, dopachrome, adrenochrome and noradrenochrome, derived from dopamine, dopa, adrenaline and noradrenaline respectively, are efficiently conjugated with glutathione in the presence of human glutathione transferase (GST) M2-2. The oxidation product of adrenaline, adrenochrome, is less active as a substrate for GST M2-2, and more

efficiently conjugated by GST M1-1. Evidence for expression of GST M2-2 in substantia nigra of human brain was obtained by identification of the corresponding PCR product in a cDNA library. Glutathione conjugation of these quinones is a detoxication reaction that prevents redox cycling, thus indicating that GSTs have a cytoprotective role involving elimination of reactive chemical species originating from the oxidative metabolism of catecholamines. In particular, GST M2-2 has the capacity to provide protection relevant to the prevention of neurodegenerative diseases.

## INTRODUCTION

Oxidative processes can lead to cellular toxicity and give rise to disease in humans [1]. Toxic quinone metabolites of catecholamines may have a key function in the production of reactive oxygen species with various pathological consequences, including apoptosis [2]. For example, the pathogenesis of Parkinson's disease and schizophrenia is characterized by degeneration of the dopaminergic neurons in the nigro-striatal and mesolimbic systems, possibly involving oxidative processes [3,4]. Furthermore, adrenochrome, the oxidation product of adrenaline (epinephrine), is neurotoxic and has psychotomimetic properties [4]. The degenerative processes may involve reactive oxygen species, which, for example in the dopaminergic system, can arise from oxidation of dopamine to an *o*-quinone and its subsequent product, aminochrome (2,3-dihydroindole-5,6-dione), which in turn may elicit redox cycling. Key reactions promoting the formation of reactive oxygen species, with a consequent prooxidant effect, include one-electron reduction of quinones catalysed by flavoproteins, such as NADPH:cytochrome *P*-450 reductase, and the subsequent reaction between the formed semiquinone radical and dioxygen [5]. One-electron reduction accompanied by autoxidation and redox cycling in the presence of dioxygen contributes greatly to the toxicity characterizing many quinones, including those formed from catecholamines such as dopa, dopamine, adrenaline and noradrenaline (nor-epinephrine) [5–7].

The present work shows that human glutathione transferases (GSTs) [8], in particular GST M2-2, catalyse the formation of

glutathione conjugates of *o*-quinones derived from physiologically important catecholamines. In addition, the presence of GST M2-2 in the substantia nigra of human brain is demonstrated. The results strongly suggest a significant neuroprotective role of GSTs.

## EXPERIMENTAL

## Chemicals

Dopamine was from Fluka AG (Buchs, Switzerland) and adrenaline, noradrenaline, D,L-dopa, and GSH were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

## Preparation of GSTs

Human GSTs A1-1, A2-2, M1-1 (allelic variant b), M2-2, M3-3 and P1-1 (allelic variant Ile 105) (for nomenclature see [9]) were obtained by heterologous expression in *Escherichia coli* and purified by affinity chromatography (cf. [10]). Detailed descriptions of the molecular cloning and characterization of GST A4-4 (I. Hubatsch, M. Ridderström and B. Mannervik, unpublished work) and GST T1-1 (P. Jemth and B. Mannervik, unpublished work) are available from the authors. Protein concentration was determined by the method of Bradford [11].

## Synthesis of aminochrome and related compounds

Oxidation of dopamine, D,L-dopa, adrenaline and noradrenaline to the corresponding *o*-quinone with the amino chain cyclized

Abbreviation used: GST, glutathione transferase.

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were performed as described previously [7,12–14]. The oxidizing agent was the  $Mn^{3+}$ -pyrophosphate complex prepared as described by Archibald and Fridovich [15].

### Assay conditions

The standard assay system for measuring conjugation of *o*-quinone with GSH catalysed by GSTs contained 0.1 M sodium phosphate, pH 6.5, 1 mM glutathione and 300  $\mu$ M *o*-quinone at 30 °C. The reaction was started by addition of GST. The reaction was recorded by monitoring the decrease of absorption at 475, 475, 480 and 483 nm of aminochrome, dopachrome, adrenochrome and noradrenochrome respectively. The reaction rate was calculated by using the extinction coefficient for aminochrome (3058  $M^{-1}\cdot cm^{-1}$ ), dopachrome (4770  $M^{-1}\cdot cm^{-1}$ ), adrenochrome (4470  $M^{-1}\cdot cm^{-1}$ ) and noradrenochrome (5502  $M^{-1}\cdot cm^{-1}$ ) respectively [7,12–14]. The steady-state kinetic parameter  $k_{cat}/K_m$  was determined at low aminochrome concentrations, i.e.  $\ll K_m$ , from the expression  $v = [E]\cdot[aminochrome]\cdot k_{cat}/K_m$ . The second-order rate constant,  $k_2$ , for the uncatalysed reaction was determined from  $v = k_2\cdot[GSH]\cdot[aminochrome]$ . The measurements were carried out with 1 mM glutathione at pH 6.5 and 7.2 in 0.1 M sodium phosphate at 30 °C.

### Cloning of cDNA encoding GST M2-2

Oligonucleotide primers 5'-GAGGAGGAATTCATGCCCAT-GACTGGGGTAC-3' (forward) and 5'-GAGGAGGTGC-ACTCATTACTTGTGCCCCAGACAGCCAT-3' (reverse), based on the published DNA sequence [16], were used for PCR to isolate cDNA encoding GST M2-2 from a human substantia nigra cDNA library (Stratagene, La Jolla, CA, U.S.A.). PCR amplification of GST M2-2 cDNA was performed using the following temperature cycle repeated 30 times: 94 °C, 1 min, 60 °C, 2 min, 72 °C, 2 min. The isolated DNA fragment was subcloned into the *EcoRI*–*SalI* sites of pGEM-3Z. The sequence of the isolated cDNA was determined by dideoxynucleotide sequencing [17].

### RESULTS AND DISCUSSION

Oxidation of catecholamines to the corresponding *o*-quinones and their subsequent reduction to reactive species, which can induce redox cycling, has been postulated to be involved in neurodegeneration in the mesolimbic and nigro-striatal systems, and thus in the etiology of schizophrenia and Parkinson's disease respectively [3,5].

The conjugation of cyclized catechol *o*-quinones with glutathione was studied using pure human GSTs. GSTs were found to catalyse the conjugation of glutathione with aminochrome, dopachrome, adrenochrome and noradrenochrome, the oxidized *o*-quinone metabolites of dopamine, dopa, adrenaline and noradrenaline respectively (Table 1). However, marked differences in the specific activities of the different GSTs were noted. GST M2-2 of the Mu class was by far the most active enzyme with all *o*-quinones tested, except for adrenochrome. Enzymes from classes Alpha (GSTs A1-1, A2-2 and A4-4), Pi (GST P1-1), and Theta (GST T1-1) displayed low or negligible activities. With aminochrome, dopachrome and noradrenochrome, GST M2-2 was 50–200-fold more active than GST M1-1, the second most efficient enzyme. In the case of adrenochrome, GST M1-1 was 1.5-fold more active than GST M2-2, but the activity of GST M1-1 was still low in comparison with the activities recorded for GST M2-2 and the other *o*-quinone substrates (Table 1). It should be noted that the high activities determined with GST

**Table 1** Glutathione conjugation of cyclized *o*-quinones of catecholamines catalysed by human GSTs

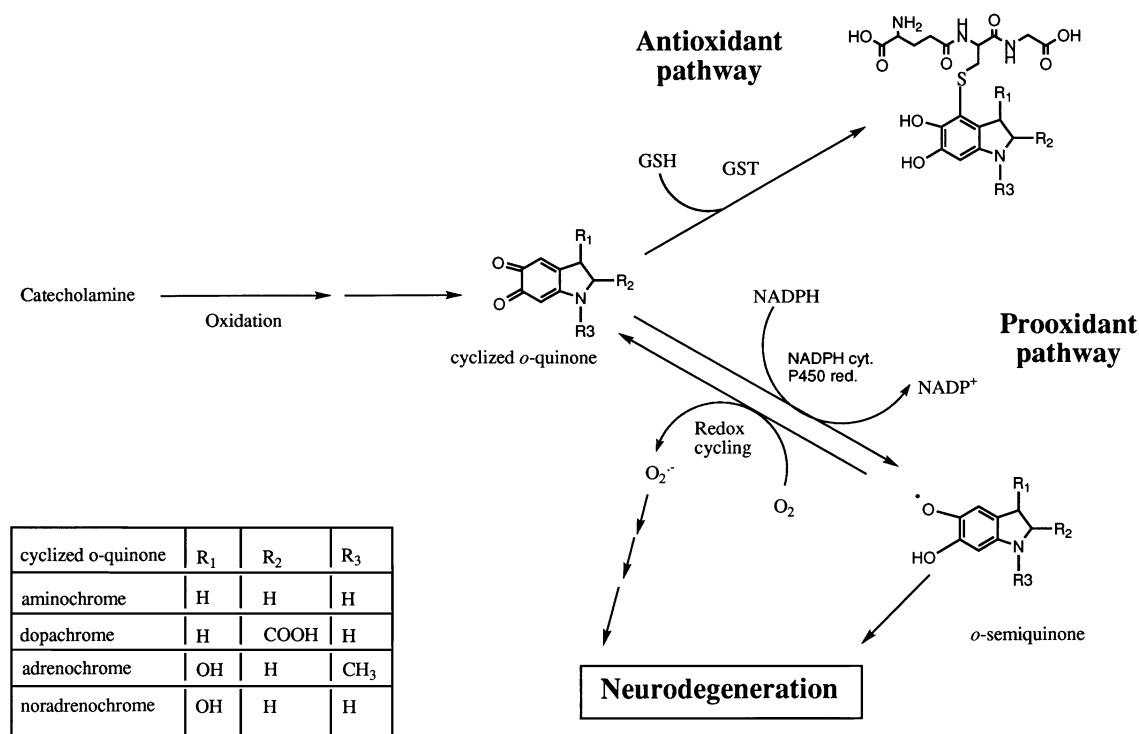
The assay system contained 300  $\mu$ M *o*-quinone (aminochrome, dopachrome, adrenochrome or noradrenochrome), 1 mM GSH and purified human recombinant GSTs in 0.1 M sodium phosphate, pH 6.5, at 30 °C. The reaction rate was monitored by recording the decrease in the absorbance of *o*-quinone in the spectral region 475–483 nm (see the Experimental section). The initial rate of the non-enzymic reaction in the presence of 1 mM GSH was  $8.6 \pm 0.2$ ,  $0.9 \pm 0.2$ ,  $0.7 \pm 0.3$  and  $2.9 \pm 0.7$   $\mu$ M/min for aminochrome, dopachrome, adrenochrome and noradrenochrome respectively. The non-enzymic rates were subtracted in the calculation of the specific activities. ND, not determined. The aminochrome data are largely from [20].

GST	Specific activity (nmol/min per mg of protein)			
	Aminochrome	Dopachrome	Noradrenochrome	Adrenochrome
A1-1	37 $\pm$ 2	18 $\pm$ 8	45 $\pm$ 17	4 $\pm$ 3
A2-2	31 $\pm$ 2	21 $\pm$ 1	ND	ND
A4-4	0	0.6 $\pm$ 0.2	2 $\pm$ 0.6	4 $\pm$ 3
M1-1	763 $\pm$ 90	1200 $\pm$ 130	470 $\pm$ 60	975 $\pm$ 80
M2-2	148 000 $\pm$ 1300	64 000 $\pm$ 6000	75 000 $\pm$ 16 000	630 $\pm$ 110
M3-3	143 $\pm$ 22	42 $\pm$ 2	ND	ND
P1-1	81 $\pm$ 26	36 $\pm$ 2	17 $\pm$ 2	21 $\pm$ 8
T1-1	0	0	0	0

M2-2 are matched only by those obtained with the most active GST substrates, such as 1-chloro-2,4-dinitrobenzene [8].

The catalytic efficiency of GST M2-2,  $k_{cat}/K_m$ , for aminochrome at a physiologically relevant concentration (1 mM, essentially saturating the enzyme) was  $21 \times 10^4 M^{-1}\cdot s^{-1}$  at pH 6.5 and  $67 \times 10^4 M^{-1}\cdot s^{-1}$  at pH 7.2. Comparison with the second-order rate constant,  $k_2$ , for the uncatalysed reaction ( $0.47 M^{-1}\cdot s^{-1}$  at pH 6.5 and  $1.1 M^{-1}\cdot s^{-1}$  at pH 7.2) showed an enzymic rate enhancement of (4–6)  $\times 10^3$ -fold. According to calculations for estimated physiological conditions in the human brain, using the concentration 2  $\mu$ g of GST M2-2 per mg of protein (J. D. Rowe and I. Listowsky, personal communication) and cellular glutathione concentration in the range 1–5 mM, the enzymic rate is > 1000-fold higher than the non-enzymic glutathione conjugation of aminochrome.

It is well established that glutathione conjugates of some quinones, such as 2-hydroxy-*p*-benzoquinone, 2,3-epoxy-*p*-benzoquinone and 1,4-naphthoquinone derivatives, undergo redox cycling in the presence of dioxygen [18,19]. With such compounds, glutathione conjugation has a prooxidant character which may be detrimental to the cell. In contrast, we have recently reported that the GST-catalysed conjugation of one of the catecholamine-derived *o*-quinones, aminochrome, leads to the formation of 4-*S*-glutathionyl-5,6-dihydroxyindoline [20], a reaction which has an antioxidant character. The glutathione conjugate was shown to be stable in the presence of dioxygen, superoxide radicals and hydrogen peroxide, and therefore does not support redox cycling. The stability of the conjugate contrasts with the lability of the product of one-electron reduction of aminochrome (*o*-semiquinone) catalysed by NADPH: cytochrome *P*-450 reductase. The *o*-semiquinone is oxidized in the presence of dioxygen, giving rise to redox cycling between aminochrome and *o*-semiquinone, accompanied by the formation of superoxide radicals (Figure 1). In addition, the antioxidant enzymes superoxide dismutase and catalase play a pro-oxidant role during aminochrome reduction, catalysed by NADPH: cytochrome *P*-450 reductase, by increasing the formation of reactive oxygen species as a consequence of an increased autoxidation rate [5]. In contrast, the conjugation of aminochrome with glutathione effectively competes with the one-electron reduction



**Figure 1** Possible pathways of pro-oxidant and antioxidant metabolism of *o*-quinones derived from catecholamines

The pro-oxidant pathway resulting in degenerative processes in the catecholamine-containing cellular systems is a consequence of the formation of reactive oxygen species derived from the one-electron reduction of *o*-quinones (aminochrome, dopachrome, adrenochrome and noradrenochrome) to *o*-semiquinones. *o*-Semiquinones are reoxidized in the presence of dioxygen, giving rise to redox cycling, in which very small amounts of catechol *o*-quinones can generate large amounts of reactive oxygen species. An important and potent antioxidant mechanism is the reductive conjugation of *o*-quinones to yield an unreactive glutathione conjugate, since this conjugate is resistant to oxidation by dioxygen, superoxide radicals and hydrogen peroxide. This antioxidant pathway may therefore play a neuroprotective role in the prevention of catecholamine-dependent formation of reactive oxygen species.

catalysed by NADPH: cytochrome *P*-450 reductase, and therefore inhibits the formation of reactive oxygen species [20] (Figure 1).

The glutathione conjugates of the additional *o*-quinones derived from catecholamines are also resistant to reoxidation in the presence of dioxygen. No evidence for the visible spectral absorption maximum characterizing quinones was detected within 40 min under aerobic conditions (at pH 6.5). Although the stability of the glutathione conjugates of the catecholamine products may require more detailed studies, we conclude that the reactions catalysed by GST serve an antioxidant function that provides cellular protection.

A possible neuroprotective role of reduced glutathione has been proposed. The level of reduced glutathione in substantia nigra of patients with Parkinson's disease has been found to be decreased [21]. Dopamine and L-dopa have been reported to induce apoptosis in thymocytes, catecholaminergic PC12 cells and sympathetic neurons [22–24]. It seems plausible that the apoptotic activity of catecholamines can be related to their oxidation to corresponding *o*-quinones and their subsequent reduction to reactive species. Furthermore, thiol-containing compounds (GSH, *N*-acetylcysteine and dithiothreitol) prevent dopamine-induced apoptosis [2]. Therefore, the protective effect of glutathione against catecholamine-induced apoptosis may be mediated by GSTs, in particular isoenzymes M2-2 and M1-1 of the Mu class. Thus, a physiological role of glutathione involving reductive conjugation of catecholamine *o*-quinones is strongly indicated in the protection of cells of the nervous system against degenerative processes.

The neuroprotective role of GST M2-2 suggested in this study is dependent on the presence of this enzyme in relevant regions of the human brain, i.e. regions where cell damage is observed in diseases such as schizophrenia and Parkinson's disease. GST M2-2 has recently been identified as one of the most abundant GSTs in human brain (J. D. Rowe and I. Listowsky, personal communication). Evidence for the expression of GSTs M3-3, M4-4 and M5-5 has previously been published [8]. In the present study we have cloned GST M2-2 from a human substantia nigra cDNA library, thus demonstrating the expression of this enzyme in the relevant region of the brain. The cDNA isolated encoded a protein identical with GST M2-2 cDNA previously isolated from muscle [16]; a single nucleotide difference in the translated region of the cDNA caused a silent T → C transition in the third position of the codon for Asn-74. Consequently, our results suggest that GST M2-2 is an important protective enzyme in the brain, although we cannot disregard the possible contribution of other GSTs with lower specific activities in the metabolism of *o*-quinones generated from catecholamines.

Thus, the results demonstrate that GSTs may contribute to cellular antioxidant defence, protecting tissues against the toxic effects of quinones. It remains to be demonstrated in further detail at the protein level, that the cellular localization of the most active isoenzymes is compatible with their proposed role in preventing the degenerative processes leading to Parkinson's disease, schizophrenia and other pathological conditions.

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